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IN 10/008,523

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jiri Snaidr	Examiner:	Sally A. Sakelaris
Serial No.:	10/008,523	Group Art Unit:	1634
Filed:	November 7, 2001	Docket No.:	235.017US1
Title:	METHOD OF DETECTING MICROORGANISMS IN A SAMPLE		

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**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

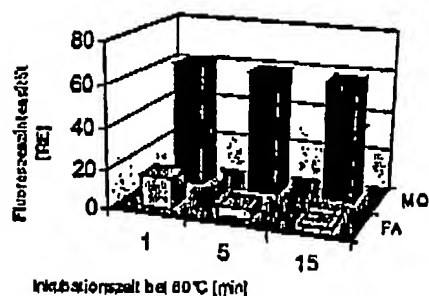
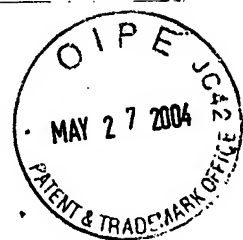
I, Dr. Jiri Snaidr, declare as follows:

1. I am the inventor of the claims of the present application and make this Declaration in support of the patentability of the claims of this application as amended in the Amendment which accompanies this Declaration.
2. The claims of the above-identified application are directed to methods to detect microorganisms which employ a particular separation solution, e.g., one having water, 0.001-0.01 M Tris/HCl, pH 9.0 +/- 2.0, DMSO or 1X SSC.
3. As described at page 8, line 25-page 9, line 13 of the specification, experiments were performed with the following separation solutions at 80°C: 0.01 M Tris-HCl, pH 9.0, water, 1 X SSC, pH 10.0, DMSO, and formamide; and at 100°C: 0.01 M Tris-HCl, pH 9.0, water, and formamide. It is disclosed that all of the non-formamide separation solutions provide a better signal than a formamide separation solution (page 8, line 34-page 9, line 3 and page 9, lines 6-11).
4. In addition, Cy3-labeled oligonucleotides were diluted in water or formamide and incubated at 80°C for up to 15 minutes (Figure 1). The fluorescent signal obtained from oligonucleotides diluted in formamide was about 80% lower than the signal obtained from oligonucleotides diluted in water.

5. Moreover, the signal from Cy3-labeled oligonucleotides diluted in 0.01 M Tris-HCl, pH 9, and incubated at 80°C was similar to the signal obtained with DMSO (Figure 2).
6. Thus, the use of separation solutions within the scope of the claims to denature target hybridized probes having a detectable signal yields a stronger signal than the use of a formamide separation solution to denature those probes.
7. I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

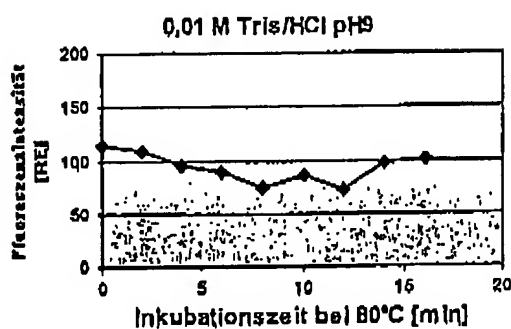
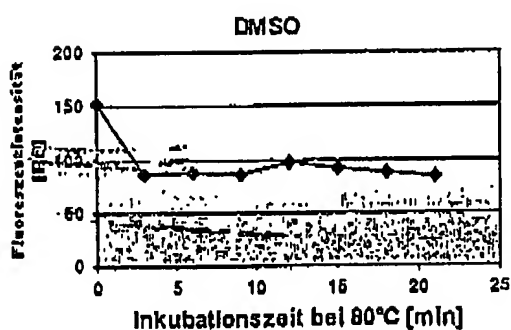
Dated: \_\_\_\_\_

By: \_\_\_\_\_  
Dr. Jiri Snaidr



Measurement of EUB338-Cy3 [50ng/110 $\mu$ l] diluted in H<sub>2</sub>O or formamide, respectively, in the spectral fluorometer, HVL 400, MQ means dist. H<sub>2</sub>O.

Fig 1



Measurement of NonEUB338-Cy3 [50ng/110 $\mu$ l] diluted in DMSO or 0,01M HCl pH 9), respectively.

Fig 2